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Growth factors play an important role in the development and growth of human prostate cancer (CaP). In normal prostatic cells, Transforming Growth Factor-α (TGFα) stimulates while Transforming Growth Factor-β (TGFβ) inhibits cell growth. To study the role that these growth factors play in CaP, we have created transgenic mouse models where the over expression of TGFα occurs (MT-TGFα) or the TGFβ signal is lost (MT-DNIIR). Since the loss of the tumor suppressor genes p53 and RB are associated with CaP, we established LPB-Tag transgenic mice that disrupt these pathways. The transgenic mice which over express the stimulatory growth factor, TGFα, develop prostatic intraepithelial neoplasia (PIN), a precursor lesion seen in human CaP. Also, mice that cannot respond to TGFβ inhibition develop PIN. LPB-Tag mice develop PIN and eventually invasive CaP. Cross breeding the MT-TGFα and MT-DNIIR mice results in offspring rapidly developing high grade PIN. If LPB-Tag mice are bred with MT-DNIIR mice, the offspring rapidly develop CaP. These results demonstrate that combining increased expression of MT-TGFα with the loss of the TGFβ signal increases the rate of PIN development. Further, the loss of the TGFβ signal and the loss of two tumor suppressors genes are sufficient to develop CaP. We are now testing the role that loss of the TGFβ plays in progression of CaP from an androgen-dependent to androgen-independent cancer. The answers to these questions will provide insight on the disease process and possible sites for intervention to treat CaP.

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INTRODUCTION: Using the prostate-specific large probasin (LPB) promoter, Dr. Matusik's laboratory has targeted an oncogene to the prostate and developed multiple new transgenic mouse lines that reproduce a full spectrum of human prostate disease including preneoplastic lesions which are similar to human prostatic intraepithelial neoplasia (PIN), local invasive carcinoma, androgen-dependent tumor growth and androgen-independent neuroendocrine prostate cancer. Dr. Moses' laboratory have developed a new transgenic line that disrupts the growth inhibitory signal of the Transforming Growth Factor β (TGFβ). These mice develop both low grade and high grade PIN. Dr. Coffey's laboratory have begun the characterization of a transgenic line where the over expression of a growth stimulatory signal, Transforming Growth Factor α, (TGFα) results in pre neoplastic lesions in the mouse prostate. Using these new transgenic mouse lines and by developing additional mouse models, the Center will assess these pathway's role to trigger prostate cancer development, tumor progression from latent to metastatic cancer, and emergence of hormone-independent prostate cancer following androgen deprivation therapy. This analysis requires the expertise of Dr. Shappell's Pathology Core that provides quality control and the evaluation of the progressive mouse pathology compared to his knowledge of human prostate cancer. These models will assist the Center in testing new drugs for the treatment of prostate cancer. The general *hypothesis* is that changes in the inhibitory signals of TGFβ and the stimulatory signals of TGFα are fundamental during prostate carcinogenesis and progression to androgen-independent disease. The Center includes three projects and a core. **Project 1:** *The role of the TGFβ pathway in prostate cancer progression to an androgen-independent disease.* Drs. Robert J. Matusik and Susan Kasper. **Project 2:** *Tumorigenic effects of partial versus complete ablation of the TGFβ type II receptor in prostatic epithelial cells.* Dr. Harold L. Moses. **Project 3:***Tumorigenic effect of TGFα in mouse prostatic epithelial cells and therapeutic efficacy of combined blockade of EGF receptor and TGFα cleavage in mouse prostate cancer.* Dr. Robert J. Coffey. **Pathology Core:** Dr. Scott Shappell has an extensive background in a variety of rat and mouse models for prostate cancer which he can relate to human prostate cancer.

The Vanderbilt Prostate Cancer Center's (VPCC) program presents an opportunity to establish a world-class research and training program at Vanderbilt University Medical Center that will increase our understanding on the basic processes involved in the development and progression of prostate cancer. The role of growth inhibitory and stimulatory pathways in prostate cancer is being evaluated in transgenic animals. These mice are now serving as models of human prostate cancer that will be useful for preclinical prevention and treatment studies. This knowledge will increase the options that are available to the medical community for effective therapy.

PROGRESS REPORT:

Project 1: *The role of the TGFβ pathway in prostate cancer progression to an androgen-independent disease.*

Significant correlative evidence has proposed a role for TGFβ ligands in the development of the prostate and progression of prostate cancer (CaP). In humans, increasing CaP grade has been correlated with increasing levels of TGFβ1. As TGFβ1 normally inhibits prostatic cell growth, increased expression of TGFβ1 in CaP has presented a conundrum. It has been hypothesized that if the CaP cells are unable to respond to the inhibitory effects of TGFβ1 yet are over producing this potent immunosuppressor, then active expression of TGFβ1 could be a selective advantage. Further

androgen ablation therapy is human prostate cancer patients results in tumor regress but eventually the patients will fail therapy. As the human cancer progresses, a loss of the TGFβ type I and type II receptor occurs. These data suggest that the TGFβ pathway may be important both in the development of prostate cancer and is progression during the failure of therapy.

To study the role of the TGFβ pathway in prostate cancer, creating various mouse transgenic lines was proposed. The goal was to analyze the role of this pathway in the development and progression of prostate cancer to androgen independence. To do so, first we blocked a functional TGFβ pathway in the prostate. Transgenic mice were generated that express a metallothionein (MT) promoter driven truncated TβRII dominant negative (DN) mutant (MT-DNIIR)[1]. The DNIIR protein will form a dimer with the TGFβ type I receptor to block the receptors response to the ligand--TGFβ. Examination of the histology of MT-DNIIR prostates revealed focal changes in prostatic morphology at approximately 12 weeks of age that are comparable to low grade prostatic intraepithelial neoplasia (LGPIN) in humans. By 16.5 weeks of age regions of high grade prostatic intraepithelial neoplasia (HGPIN) was present in all animals examined. At 33 weeks, only one mouse prostate showed a local invasion; however, these mice develop defects in the skeleton that prevents keeping them past this age. Further, the major changes occur in the mouse dorsolateral prostate, the region most closely akin to human peripheral zone, the region that develops prostate cancer.

In rodents it is established that androgen ablation will induce almost complete prostate regression which is preceded at four days post-castration by a 40-fold increase in TGFβ1[2]. Also, the administration of TGFβ1 *in vivo* will induce prostate regression [3]. These data suggest androgen ablation induces TGFβ1 which results in prostate regression. Since the MT-DNIIR transgenic mice would block the TGFβ pathway, would the prostates in these mice regression after castration? We found that seven days post-castration does not induce regression of the prostate in MT-DNIIR mice but regression does occur after 35 days post-castration. Thus regression is delayed but not prevent. We are now investigating the possible mechanism that eventually results in prostatic regression. These results provide us with an opportunity to study the role that TGFβ1 may play in the failure of prostate cancer to hormone therapy.

In parallel, we have developed transgenic mice that targets expression of the SV40 large T antigen (Tag) to the prostate using the prostate-specific large probasin promoter (LPB). The Tag protein binds and inactivates two tumor suppressor genes, p53 and Rb, two genes that can be inactivated in late stage CaP and recent reports have identified p53 loss is some HGPIN. The LPB-Tag mice develop HGPIN by 16 weeks and some develop limited invasive cancer after 20 weeks of age. These tumors are androgen dependent for growth and regression after androgen ablation therapy (castration) [4]. Between 2 to 6 months after castration, approximately 75% of the tumors will regrow. Accurate rates of growth, regression, and regrowth are being monitored by MRI. Pathology of the regrowing tumors is now be assessed.

Bigenic males from the MT-DNIIR x LPB-Tag cross are being studied in a time course of 12-23 weeks for gross, histological, and immunohistological characteristics. The preliminary data shows that the bigenic mice developed both HGPIN and invasive prostate cancer in 100% of the animal ≥ 16 wks with both glandular and neuroendocrine differentiation. This is in sharp contract to the MT-DNIIR mice and the LPB-Tag mice which would only have HGPIN at this age. Further, metastatic Tag positive carcinomas, primarily with NE differentiation, were noted in para-aortic lymph nodes,

bone, and viscera, including liver and lung of bigenic mice ($\geq$ 50 % of mice $\geq$ 16 wks). These data demonstrate the loss of the TGFβ pathway along with a loss of two tumor suppressor genes, p53 and Rb, is sufficient to development of prostate cancer. Since the TGFβ pathway involves activation of a number of genes and a number of separate pathways, these animals are be analyzed to determine the key components involved in tumor development.

Now, using the MT-DNIIR cross LPB-Tag, which develops adenocarcinoma, we will study the effect of castration these bigenic mice. Since LPB-Tag tumors regress and MT-DNIIR prostate show a dramatic decrease in the rate of regression after castration, we expect that the tumors in the bigenic mice will regress at a slower rate and that they may progress at a faster rate to become androgen independent.

Project 2: *Tumorigenic effects of partial versus complete ablation of the TGFβ type II receptor in prostatic epithelial cells.*

Prostate glands have been harvested from mice beginning at 2 weeks of age at intervals through puberty for histology, immunohistochemistry and *in situ* hybridization. Before performing immunohistochemistry for detection of TβRII the different antibodies available were tested for specificity. Cell lines containing TβRII and lacking TβRII were used. Different fixatives such as 4% paraformaldehyde, acetone, methanol and 10% formalin were evaluated. Several TβRII antibodies, including rabbit polyclonal (Upstate Technology cat 06-227 and 6-318; Santa Cruz cat#sc-220), and goat polyclonal (cat# AF-241-NA) at concentrations of 0, 5, 10, 15 μg/ml. Positive staining was obtained in both TβRII positive and negative cells and tumor xenographs indicating that the specificity of the antibodies for immunohistochemistry was a significant problem. Further, antibodies suitable for immunohistochemistry have not been identified for either of the type I receptors, Tsk7L/ALK2 and ALK5/R4. Thus, in the absence of suitable antibodies, we will utilize *in situ* hybridization for TβRII as well the type I receptors, and these experiments are in progress. Hybridization of tissue sections has been accomplished and the emulsion coated slides are being exposed. In addition, because of progress in the tissue core with real time PCR, we will use this method to obtain better quantitation of mRNA expression for the three different receptors during prostate gland development to compare with the localization of expression obtained by in situ hybridization.

A major improvement to target expression to the transgenic mouse prostate has been accomplished by redesigning LPB promoter into a new construct that is now termed ARR_2PB [5]. This new prostate-specific ARR_2PB promoter has replaced LPB for all future experiments. Three lines of ARR_2PB-DNIIR mice have been generated by the VICC Transgenic Mouse/ES Cell Shared Resource by microinjecting a previously assembled ARR_2PB-DNIIR construct. Offspring of founder mice from the three ARR_2PB-DNIIR lines (lines A, B, and C) have been shown to have the appropriate genotype. Prostates have been harvested from the three lines of LBP-DNIIR animals, and expression of the TGFβ dominant-negative TypeII receptor (DNIIR) is currently being determined by RT-PCR and characterization of the phenotype is under way.

In the grant application, we described the generation of mice having 3 LoxP sites flanking exon 2 of the type II TGFβ receptor gene (*Tgfbr2*) and a *NeoR* cassette. Mice homozygous for this allele were found to die before birth. We described in the original application the strategy for

selectively excising the *NeoR* cassette by pronuclear microinjection of a supercoiled *Cre* expression plasmid into *Tgfbr2*$^{Lox+Neo}$ one cell embryos. This has been accomplished, and we now have *Tgfbr2*$^{floxE2/floxE2}$ mice. The homozygous mice are viable and fertile and have been crossed with three different lines of transgenic mice expressing Cre recombinase under control of three different promoters (MMTV-Cre, Ck-19-Cre, and Alb-Cre). Recombination of the *Tgfbr2* locus was obtained in the appropriate tissues in each circumstance demonstrating that the proposed experiments are feasible.

ARR_2PB-Cre mice have been created and characterized in collaboration with Dr. Pradip Roy-Burman [6]. We are presently crossbreeding the ARR_2PB-Cre-GH mice with the *Tgfbr2*$^{floxE2/floxE2}$ to generated mice that are homozygous for the floxed *Tgfbr2* locus and express the ARR_2PB-Cre. Mice with this genotype should exhibit knock out of *Tgfbr2* in prostatic epithelium.

Project 3: *Tumorigenic effect of TGFα in mouse prostatic epithelial cells and the therapeutic efficacy of combined blockade of EGF receptor and TGFα cleavage in mouse prostate cancer.*

TGFα is overproduced in human prostate cancer[7]. TGFα is one of 6 mammalian ligands that bind the Epidermal Growth Factor Receptor (EGFR) that include EGF, TGFα, heparin-binding EGF-like growth factor, amphiregulin, betacellulin, and epiregulin. They are produced in pro-forms that are inserted into the plasma membrane and cleaved by specific proteases to release the mature, soluble, fully active growth factor. Expression of these ligands has not been studied systematically in normal and malignant prostate.

Our hypothesis is that normal epithelial cell growth is regulated by a balance of stimulatory (e.g., TGFα) and inhibitory (e.g., TGFβ) factors and that cancer results, at least in part, when there is an excess of the stimulatory arm and/or a defect in the inhibitory arm. This model was formulated using skin cells in culture and validated it by establishing mouse models of breast cancer [8]. Previous studies have shown that over-expression of TGFα resulted in hyperplasia and displasia in the coagulation gland (anterior prostate) of the mouse [9]. In our hands, a single, 16-week-old transgenic mouse in which TGFα was expressed under the metallothionien promoter (MT-TGFα) was found to have prostatic intraepithilial neoplasia (PIN) lesions. We have since examined the prostates of 5 additional MT-TGFα mice at 15 weeks of age and confirmed that these mice develop PIN lesions.

To investigate whether simultaneous manipulation of both the stimulatory and inhibitory axes will enhance the development of precancerous lesions, the MT-TGFα and MT-DNIIR mice were crossbred. Transgenic mice with a metallothionein-driven dominant negative TGFβ type II receptor have also been shown to develop PIN. This was confirmed, as control animals with one or the other transgene developed PIN lesions. The bigenic MT-TGFα cross MT-DNIIR animals at 15 weeks of age were found to have PIN lesions of a higher grade than either the MT-TGFα or MT-DNIIR mice. Age-matched non-transgenic mice did not develop prostatic lesions.

Since the metallothionein promoter expresses throughout the animal, including both the epithelium and stroma of the prostate, we are also generating transgenic lines expressing TGFα under the ARR_2PB promoter, which expresses exclusively in the epithelium of the mouse prostate[5].

Comparing these lines with the MT-TGFα line will allow us to investigate involvement of EGFR signaling in epithelial-stromal interactions.

The mouse models we have generated support our hypothesis that TGFα and EGFR signaling regulate growth of prostatic epithelium and that hyper-stimulus of this growth-stimulatory axis can result in hyperplasia. The bigenic animal adds credence to a model in which the stimulatory axis including TGFα acts in balance with a growth inhibitory axis including TGFβ signaling and that alteration in both these axes can result in a greater degree of hyperplasia. Evaluation of these transgenic animals is in progress. We believe this models will prove useful tools in understanding the actions of TGFα and the EGF receptor in prostate cancer.

KEY RESEARCH ACCOMPLISHMENTS:

Project 1: *The role of the TGFβ pathway in prostate cancer progression to an androgen-independent disease.*

Task I: Characterization of MT-DNIIR-27 and MT-DNIIR-4 mice.

Dr. Tania Thomas has completed this task, she has left the University, and is preparing a manuscript to describe the results. An abstract has been presented at the Endocrine Meeting in 1999 and the AUA meeting in 2000 (see Abstracts in Appendices)

- With aging, the MT-DNIIR-27 and MT-DNIIR-4 transgenic mice show the most significant changes in the dorsolateral prostate which include the development of high grade prostatic intraepithelial neoplasia (HGPIN).
- In one MT-DNIIR-4 transgenic mouse, invasive prostate cancer developed at 33 weeks of age. However, due to skeletonal defects that these mice also develop after 30 weeks of age, we are not able to maintain the animals past this time. Therefore, all long term studies are now being carried out with the MT-DNIIR-27 transgenic line.

Task II: MT-DNIIR x LPB-Tag transgenic lines.

Mr. William Tu (MD/Ph.D. student) has the prime responsibility is to characterize the cross between the MT-DNIIR-27 with the LPB-Tag transgenic lines. Preliminary results are being presented at the AUA meeting in June 2001 (see Abstract in Appendices).

- In 16 week old MT-DNIIR-27 mice and in 16 week old LPB-Tag mice HGPIN lesions are seen in the dorsolateral prostate. When the two lines are crossed, 16 week old mice develop prostate cancer.

Task III: Progression after androgen ablation in the LPB-Tag mice.

This task was started.

- After castration and regression of the prostate tumor, we see regrowth of the LPB-Tag tumors. This study is ongoing and the data is being analyzed.

Task IV: Progression after androgen ablation in the MT-DNIIR x LPB-Tag Mice.

- To be completed between 24-30 months of the grant.

Project 2: *Tumorigenic effects of partial versus complete ablation of the TGFβ type II receptor in prostatic epithelial cells.*

Task I: Characterize TβRI and TβRII expression during prostate development in the mouse.

- Prostate glands have been harvested from mice beginning at 2 weeks of age at intervals through puberty for histology, immunohistochemistry and *in situ* hybridization.
- Characterization of available antibodies has been performed revealing the lack of a suitable antibody for TβRII and TβRII
- Tissue sections have been cut and *in situ* hybridization for TβRII as well the TβRI is in progress.

Task II: Disrupt the TGF-ß pathway specifically in epithelium with the ARR_2PB-DNIIR transgene. The LPB promoter has now been replaced by an improved version of a prostate-specific promoter termed ARR_2PB [5].

- Three lines of ARR_2PB-DNIIR transgenic mice have been established
- Characterization of these lines has begun.

Task III: Create and cross breed ARR_2PB-Cre mice with *Tgrbr2*^{floxE2} mice for complete abrogation of TGF-ß signaling.

- Mice carrying the floxed TβRII receptor have been made (*Tgfbr2*$^{floxE2/floxE2}$ mice).
- The homozygous *Tgfbr2*$^{floxE2/floxE2}$ mice are viable and fertile.
- ARR_2PB-Cre mice have been created and characterized in collaboration with Dr. Pradip Roy-Burman [6].
- Presently crossbreeding the ARR_2PB-Cre mice with the *Tgfbr2*$^{floxE2/floxE2}$ is starting. Mice with this genotype should exhibit knock out of *Tgfbr2* in prostatic epithelium.

Project 3: *Tumorigenic effect of TGFα in mouse prostatic epithelial cells and therapeutic efficacy of combined blockade of EGF receptor and TGFα cleavage in mouse prostate cancer.*

Task I: To develop and characterize ARR_2PB-TGFα transgenic mice and compare them to MT-TGFα mice.

- Our latest advance in constructs has replaced the LPB promoter with the ARR_2PB promoter [5]. Using this construct, four independent transgenic mouse lines have been generated carrying a ARR_2PB driven TGFα gene. These lines are being breed to obtain sufficient males for aging studies.

- Twenty male MT-TGFα mice have been generated for comparison with the ARR_2PB-TGFα mice and as a control for the mice in task II. Five of the MT-TGFα mice have been sacrificed at 15 and 22 weeks of age and were found to have dysplastic lesions in all lobes of the prostate.

Task II: To cross MT-TGFα mice to MT-DNIIR mice as well as to cross ARR_2PB-TGFα mice to ARR_2PB-DNIIR and/or LPB-CRE/*Tgfbr2*$^{floxE2/floxE2}$ mice.

- Twenty male bigenic MT-TGFα crossed with MT-DNIIR mice have been generated, along with equal numbers of non-transgenic mice and animals with either the MT-TGFα or MT-DNIIR transgenes alone. Five of each group of mice were sacrificed at 15 weeks of age. The non-transgenic animals were found to be free of prostatic lesions while transgenic animals showed PIN like lesions. The bigenic animals consistently showed lesions of a higher grade than those of animals with only one of the transgenes. The most significant lesions were found in the anterior and dorsal lobes.

Task III: To treat mouse prostate tumors with EGFR tyrosine kinase inhibitor and/or selective TACE inhibitor.

- To be completed between 24-36 months of the grant.

CORE: Establishment of Pathology Core Laboratory and Provision of Basic Histopathology Support:

- Acquisition of Olympus SZX9 stereo dissecting microscope, Ventana Renaissance Tissue Processor, Leica EG1160 embedding station, Surgipath Medical Industries Slide Labeler, and Shandon Finesse Microtome. Mouse tissues procured in the laboratories of Drs. Matusik, Coffey, Moses, and during interventional research protocols conducted in Dr. Shappell's laboratory are processed in the Core Lab. 1 H & E and unstained charged slides for subsequent immunohistochemistry and/or in situ hybridization (if indicated) obtained on all blocks.

- Acquisition of 5-headed Olympus BX50 microscope with upgraded objectives and dark field capacity, Nikon D-1 digital camera, 733 MHz Compaq Pentium III computer with CD burner, Gateway e-5200 PC with dual 450 MHz processor, and Gateway Solo 9300 Laptop with CD burner (primarily for digital camera support). All slides are reviewed by Dr. Richard Roberts and/or Dr. Shappell, commonly with responsible investigator from individual lab. Descriptions are recorded on spreadsheet. Images documenting pertinent pathology are obtained with the D-1 camera. Such images are stored in the Core Lab as well as provided

to individual project investigators on CDs. For final model characterization/publication, slides are reviewed blindly.

- Acquistion of Shandon Cryostat. Provision of frozen sections for CD31 immunostaining, MALDI mass spectrometry.

Adjuctive diagnostic techniques:

- Establishment of immunohistochemical protocols and application to various models, supplementing immunostaining assasys performed by individual labs, including:
    - General/model characterization: Pan cyto-keratin, High molecular weight cytokeratin, CK5, PCNA, Apo-tag, AR, Chromogranin, CD31 (including on frozen sections)
    - Antibody assays for Shappell Mouse-based research: 8-lipoxygenase, platelet 12-lipoxygenase, leukocyte 12-lipoxygenase, cyclooxygenase-2
    - Antibodies currently being investigated/validated: Laminin, N-cadherin, E-cadherin, Beta-catenin.
- Performance of ultra-structural studies on DLP/VP on LPB-Tag 12T-7f x MT-DNIIR mouse.
- Establishment of quantitative Real Time RT-PCR assays on Roche LightCycler system, utilizing cDNA standard curves with cloned templates and cDNA binding fluorescent probe SYBR green or oligo specific hybridization probes. 12 specific gene products so far, including mouse β-actin, platelet 12-LOX, leukocyte 12-LOX, TGFβRI, TGFβRII.

REPORTABLE OUTCOMES: The reportable outcomes of the Vanderbilt Prostate Cancer Center are divided into three sections: 1) Institutional Commitments and VPCC; 2) Research Projects, and 3) Pathology Core.

1) Institutional Commitments and VPCC: Due to the DOD funding of the Center, Vanderbilt University Medical Center, the Vanderbilt-Ingram Cancer Center, the Section of Surgical Sciences, and the Department of Urologic Surgery have made major institutional commitments that have allowed the scope of the Center to expand beyond the initial research projects.

Administration: Dr. Robert Matusik serves as the Director of the VPCC. The Center holds research meetings on the first Wednesday of the month. During this time, research is progress from each project is discussed. Future direction and research experiments are planned. The first Annual Retreat, which includes the Steering Committee, will be held in early September 2001. By this time, all the new staff will be in place so they can participate in this Retreat.

Budget:

- The Vanderbilt University Medical Center has provided the salary for Ms. Debbie Thompson to serve as an administrative assistant to the VPCC.

- The Vanderbilt-Ingram Cancer Center has provided $200,000/ year as support for operating expenses of the Center, for equipment, secretary (Ms. Lisa Howell) and pilot projects to expand the research endeavour.

- The Department of Urologic Surgery has provided the start-up funds to recruit Dr. Simon Hayward as a new faculty member.

- The Department of Urologic Surgery is also funding a Urologic Oncology Fellowship Training program within the Center. Dr. Naoya Masumori (MD/PhD) was the first Urologist funded by this program. He returned to his clinical position at Sapporo, Japan in February 2001. Dr. Jen (MD/PHD) is a Urologist who will begin this program in May 2001.

Space for VPCC:

Laboratory Space

- The Vanderbilt University Medical Center and the Section of Surgical Sciences has provide new laboratory space for Dr. Simon Hayward and Dr. Susan Kasper. Additional laboratory space has been provided for Dr. Matusik. The laboratory space has increased by 2168 sq ft. from the previous1533 sq. ft. to new total of 3701 sq. ft. (see floor plan, Appendices)

Office Space for Faculty

- New offices are proposed for the VPCC (for a total of 596 sq. ft.). This space will undergo renovation and should be ready by July 2001. The Section of Surgical Sciences and the Department of Urologic Surgery are covering the cost for this renovation. Three offices will be for Dr. Matusik, Director, Dr. Kasper, and Dr. Hayward. A fourth office will be for Ms. Lisa Howell, secretary for the Center. The offices are near the entranceway to the research laboratories of the VPCC (see floor plan, Appendices).

Office Space for Post-doctoral fellows

- Two offices (AA-1326 and AA-1324 for a total of 136 sq. ft.) will be provided for post-doctoral fellows. These offices will have to be shared but they will provide space for the post-doctoral fellows to work on data and write manuscripts (see floor plan, Appendices).

Conference Room

- A conference room (A-1307) shared with the Department of Urologic Surgery is provide for laboratory meetings and lectures (see floor plan, Appendices).

New faculty, post-doctoral fellows,students: In the review of our application, the committee recommended that more junior faculty and post-doctoral fellows should be involved in the research. We are making major efforts to add more junior investigators to the VPCC. The following positions have now been filled.

New Faculty

- Dr. Simon Hayward has been recruited for a tenure-track Assistant Professor position in the Department of Urologic Surgery (see Curriculum Vitae, Appendices). His future graduate students would complete their Ph.D. degree program under the guidelines of the Department of Cancer Biology, his secondary appointment. Dr. Hayward's research program will focus on prostate Tumor/Host interactions. His laboratory space (AA-1309) is assigned within the VPCC space (see Floor plan, Appendices).

- Dr. Susan Kasper has been promoted from Research Assistant Professor to a tenure-track Assistant Professor in the Department of Urologic Surgery (see Curriculum Vitae, Appendices). Her future graduate students would complete their Ph.D. degree program under the guidelines of the Department of Cancer Biology, her secondary appointment. Dr. Kasper's research will develop a new program on prostate cancer progression. Her laboratory space (AA-1315) is assigned within the VPCC space (see Floor plan, Appendices).

- Dr. Richard Roberts, M.D., Ph.D., Research Instructor and Molecular Pathology Fellow. Involved in the review of mouse pathology slides, image acquisition and storage, establishing Pathology Core immunohistochemistry, EM studies, and actively involved in translational research including tumor angiogenesis. His involvement with the characterization of the animal models of prostate cancer will help translate his research into new treatments for prostate cancer.

Post-doctoral Fellows

- Dr. Shane Cutler was recruited by Dr. Coffey's laboratory (see Curriculum Vitae, Appendices). He is currently working on Project 3 to study the role of TGFα in prostate tumor development.

- Dr. Ren Jie Jin will arrive by June 1, 2001. He is a trained Urologist from China that has also completed a Ph.D. from Seoul National University, Korea (see Curriculum Vitae, Appendices). Dr. Jin will work with Dr. Matusik's laboratory on Project 1 and on the LPB-Tag transgenic animal models. He will be a new recruit to the Urology Fellowship Training Program

- Mr. Janni Mirosevich will arrive September 1, 2001. He will complete all requirements for his Ph.D. by July 2001 (see Curriculum Vitae, Appendices). Mr. Mirosevich will study gene expression on Project 1.

- Ms. Tiina Pitkänen-Arsiola will arrive in July, 2001, soon after she competes her requirements for her Ph.D. (see Curriculum Vitae, Appendices). Ms. Pitkänen-Arsiola will work with Dr. Kasper's laboratory to study progression in prostate cancer from an androgen-dependent to an androgen-independent disease.

Students

- Mr. William Tu is a MD/Ph.D. student at Vanderbilt working for Dr. Matuisk. (see Curriculum Vitae, Appendices). He is studying how combining the disruption of the TGFβ pathway and the p53/RB pathway results in developing adenocarcinoma in Project 1.

2) Research Projects: Two manuscripts are now in preparation on the role that the TGFβ pathway plays in developing prostate cancer. A number of abstracts have been presented. Also, as a result of this work, symposium lecture at meetings have resulted.

Published Abstracts

- Thomas, TZ, Shappell S, Sohn PC, Kasper S, Matusik RJ, Moses HL, Serra RA. Expression of a truncated, kinase deficient TGFβ Type II receptor in the mouse prostate. 81st Annual Meeting of The Endocrine Society, 1999.

- Thomas TZ, Shappell S, Kasper S, Serra RA, Moses HL, and Matusik RJ. Disruption of the TGFβ pathway in transgenic mice prevents castration-induced prostatic regression. The American Urological Association 95th Annual Meeting, April 29-May 4, 2000. Atlanta, Georgia.

- Tu WH, Thomas TZ, Masumori N, Tsukamoto T, Kasper S, Roberts RL, Moses HL, Shappell SB, Matusik RJ. Role of TGF-β pathway in prostate carcinogenesis. The American Urological Association 96th Annual Meeting, June 2-7, 2001. Anaheim, California.

- Robert J. Matusik, William H. Tu, Tania Z. Thomas, Naoya Masumori, Susan Kasper, Richard L. Roberts, Rosa Serra, Scott B. Shappell, and Harold L. Moses, TGF-β and Prostate Cancer. 83st Annual Meeting of The Endocrine Society, June 20-23, 2001. Denver, Colorado.

Symposium Lectures

- Dr. Matusik have been invited to present this data in the at the 83st Annual Meeting of The Endocrine Society in June 2001.

- Dr. Matusik has been invited to present his work at the NIH sponsored MMHCC Workshop on *Transgenic Models for Prostate Cancer* in October 2001.
- Dr. Moses has been invited to present his work at the NIH sponsored MMHCC Workshop

on *Transgenic Models for Prostate Cancer* in October 2001.

- Dr. Shappel will Chair the Pathology Workshop held in concert with NIH sponsored MMCCC Workshop on *Transgenic Models for Prostate Cancer* in October 2001.

Personnel:
The personnel of the VPCC include those supported by the DOD award, institutional commitments, and individuals that may be on trainee awards. Listed below are only individuals supported directly by the DOD award over the fiscal year covered by this report.

PROJECT 1:	
Robert J. Matusik, Ph.D.	PI and Director
Susan Kasper, Ph.D.	Co-Investigator
Tania Dickson, Ph.D.	Research Fellow
PROJECT 2:	
Harold L. Moses, MD	PI
Agnes Gorska	Research Tech Senior
Mary Aakre	Research Tech Senior
Anna Chytil	Research Tech Senior
PROJECT 3:	
Robert J. Coffey, MD	PI
Lu Min, Ph.D.	Research Fellow
Gelina Bogatcheva	Research Assistant III
PATHOLOGY CORE:	
Scott B. Shappell, MD, Ph.D.	PI
Richard L. Roberts, Ph.D.	Research Instructor and Fellow
Cathy Hibbs-Brown	HistoTech
Suzanne Manning	Research Assistant III

3) Pathology Core: Dr. Scott Shappell is director of the Pathology Core. The key accomplishment have been listed above. Because of Dr. Shappell expertise with human prostate cancer and mouse prostate cancer models, he has been chosen by the NCI funded Mouse Models of Human Cancer Consortium (MMHCC) to Chair a workshop on transgenic mouse prostate cancer models. This Workshop will establish the NCI standards for the characterization/validation of all mouse models for prostate cancer.

CONCLUSIONS:

Substantial progress has been made on the three individual grants and in the establishment of the Pathology Core. In addition, Vanderbilt University Medical Center, Section of Surgical

Sciences, Department of Urologic Surgery, and the Vanderbilt-Ingram Cancer Center have meet their commitments to the DOD Center grant which are beyond the initial research projects allowing use to expand the program as a new Vanderbilt Prostate Cancer Center.

REFERENCES:

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2. Kyprianou N, Isaacs JT: Expression of transforming growth factor-beta in the rat ventral prostate during castration-induced programmed cell death. Molecular Endocrinology 3:1515, 1989

3. Martikainen P, Kyprianou N, Isaacs JT: Effect of transforming growth factor-beta 1 on proliferation and death of rat prostatic cells. Endocrinology 127:2963, 1990

4. Kasper S, Sheppard PC, Yan Y, Pettigrew N, Borowsky AD, Prins GS, Dodd JG, Duckworth ML, Matusik RJ: Development, Progression And Androgen-Dependence of Prostate Tumors in Transgenic: A Model For Prostate Cancer. Laboratory Investigation 78:319, 1998

5. Zhang J, Thomas TZ, Kasper S, Matusik RJ: A small composite probasin promoter confers high levels of prostate-specific gene expression through regulation by androgens and glucocorticoids in vitro and in vivo. Endocrinology 141, 4698, 2000.

6. Wu X, Wu J, Huang J, Powell WC, Zhang J, Matusik RJ, Sangiorgi FO, Maxson RE, Sucov HM, Roy-Burman P: Generation of a prostate epithelial cell-specific Cre transgenic mouse model for tissue-specific gene ablation. Mech.Dev. 101:61, 2001

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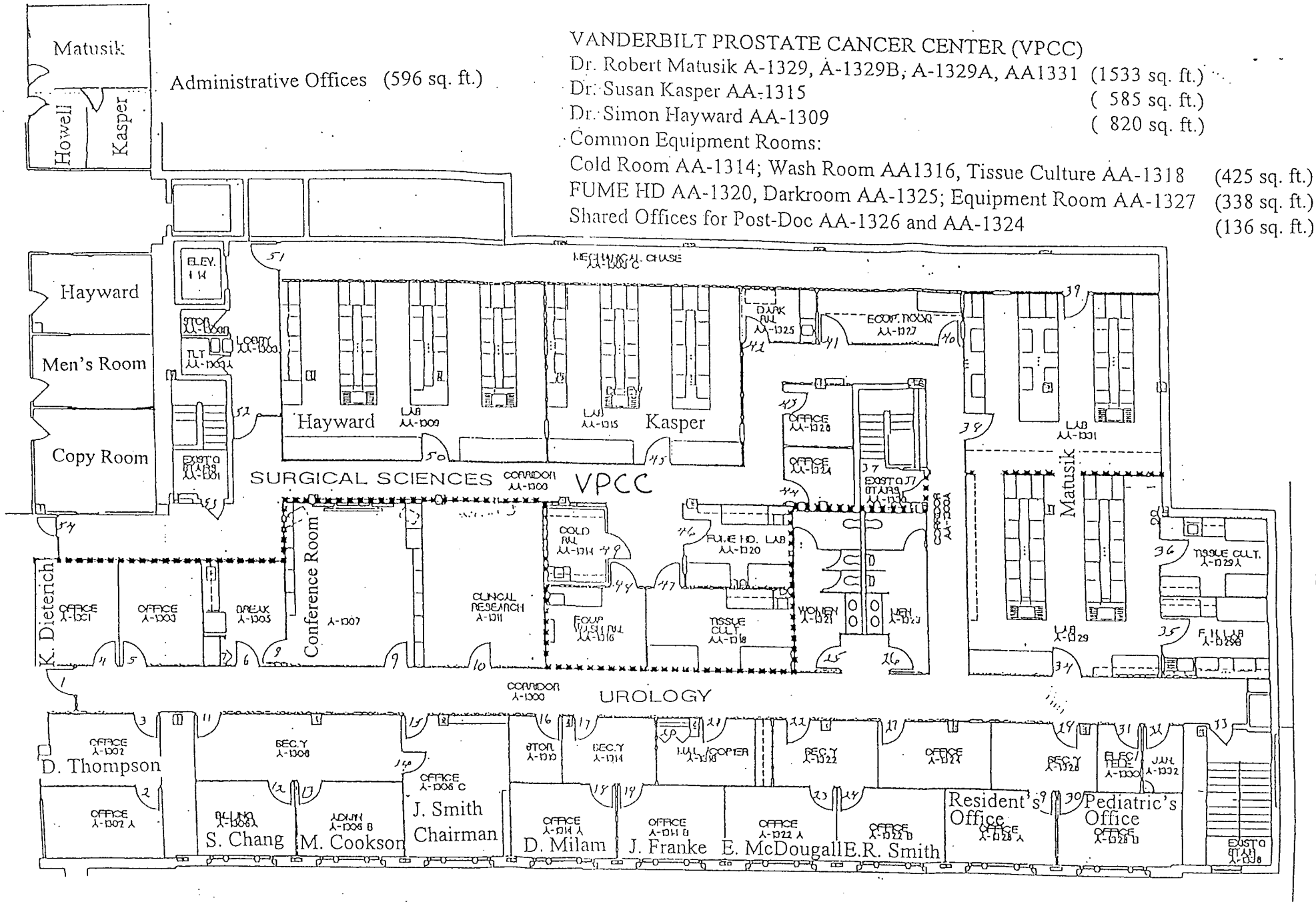
8. Pierce DF, Jr., Gorska AE, Chytil A, Meise KS, Page DL, Coffey RJ, Jr., Moses HL: Mammary tumor suppression by transforming growth factor beta 1 transgene expression. Proc.Natl.Acad.Sci.U.S.A. 92:4254, 1995

9. Sandgren EP, Luetteke NC, Palmiter RD, Brinster RL, Lee DC: Overexpression of TGF alpha in transgenic mice: induction of epithelial hyperplasia, pancreatic metaplasia, and carcinoma of the breast. Cell 61:1121, 1990

APPENDICES:

Vanderbilt Prostate Cancer Center: Offices and Laboratory Space.



DEPARTMENT OF UROLOGY/SURGICAL SCIENCES
CORRIDOR A-1300 AND AA-1300
VANDERBILT UNIVERSITY MEDICAL CENTER

Abstracts:

1) Thomas, TZ, Shappell S, Sohn PC, Kasper S, Matusik RJ, Moses HL, Serra RA. Expression of a truncated, kinase deficient TGFβ Type II receptor in the mouse prostate. 81st Annual Meeting of The Endocrine Society, 1999.

2) Thomas TZ, Shappell S, Kasper S, Serra RA, Moses HL, and Matusik RJ. Disruption of the TGFβ pathway in transgenic mice prevents castration-induced prostatic regression. The American Urological Association 95th Annual Meeting, April 29-May 4, 2000. Atlanta, Georgia.

3) Tu WH, Thomas TZ, Masumori N, Tsukamoto T, Kasper S, Roberts RL, Moses HL, Shappell SB, Matusik RJ. Role of TGF-β pathway in prostate carcinogenesis. The American Urological Association 96th Annual Meeting, June 2-7, 2001. Anaheim, California.

4) Robert J. Matusik, William H. Tu, Tania Z. Thomas, Naoya Masumori, Susan Kasper, Richard L. Roberts, Rosa Serra, Scott B. Shappell, and Harold L. Moses, TGF-β and Prostate Cancer. 83st Annual Meeting of The Endocrine Society, June 20-23, 2001. Denver, Colorado.

OR19-3

EXPRESSION OF A TRUNCATED, KINASE DEFICIENT TGFβ TYPE II RECEPTOR IN THE MOUSE PROSTATE.

T. Z. Thomas,*[1] S. Shappell,[2] P. C. Sohn,[3] S. Kasper,[1] R. J. Matusik,[1,3] H. L. Moses,[3] R. A. Serra.[3] [1]Depart of Urologic Surgery, [2]Dept of Pathology, [3]Vanderbilt Cancer Center, Vanderbilt University Medical Center, Nashville, TN

Early reports have noted increased expression of TGFβ1 and loss of TGFβ receptor type I (TβRI) and II (TβRII) expression with increasing cancer grade in human prostate cancer (PCa). Although the TGFβ superfamily has been implicated in the progression of human PCa, it's role in the development and progression of this disease has not been addressed in transgenic animal models. We have generated transgenic mice that express a metallothionein (MT) promoter driven truncated TβRII which results in a dominant negative (DN) mutant that can form a heteromeric complex with the endogenous TβRI. The prostates from two different founder lines (MT-DNIIR-4 and MT-DNIIR-27) were examined for transgene expression by *in situ* hybridization and for histopathological changes associated with loss of TGFβ function. *In situ* hybridization, using a transgene specific cDNA probe, showed transgene expression in the ventral (VP), dorsolateral (DLP) and anterior prostate (AP). The expression of the transgene in MT-DNIIR-27 mice was low at 7 weeks but increased by 16.5 weeks of age. Transgene expression was similar in MT-DNIIR-4 mice, however the levels were higher. Histological examination of MT-DNIIR-27 prostates revealed focal changes in prostatic morphology at approximately 12 weeks of age that are comparable to low grade prostatic intraepithelial neoplasia (LGPIN) in humans. By 16.5 weeks of age regions of high grade prostatic intraepithelial neoplasia (HGPIN) are present in all animals examined. MT-DNIIR-4 mice at 33 weeks show regions of HGPIN with local invasion, increased thickening of the basement membrane/fibromuscular stroma, as well as regions of cribriform and solid architecture accompanied with comedo necrosis which is reminiscent of intraductal carcinoma (IDCa). IDCa is believed to represent the intraductal spread of established carcinoma, and has been correlated with higher Gleason grade, higher tumor volume and poor prognosis. Therefore the MT-DNIIR mice show pre-neoplastic changes mimicking those observed in human prostate disease. Although the origin of human premalignant lesions remains unknown, this data suggests that disruption of the TGFβ pathway may be the initiating event(s) in mouse and human prostatic neoplasia.

Supported by J.T. Martell; J. Davis; and Ingram Charitable Funds.

Program & Abstracts

81st Annual Meeting

June 12-15, 1999 San Diego, California

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DISRUPTION OF THE TGFβ PATHWAY IN TRANSGENIC MICE PREVENTS CASTRATION-INDUCED PROSTATIC REGRESSION.

Tania Z. Thomas, Scott Shappell, Susan Kasper, Rosa A. Serra, Harold L. Moses, Robert J. Matusik. Cincinatti, OH; Nashville, TN. (Presented by Tania Z. Thomas)

INTRODUCTION AND OBJECTIVES: Early reports have noted increased expression of TGFβ1 and loss of TGFβ receptor type I and type II (TβRII) expression with increasing prostate cancer (PCa) grade. We have generated transgenic mice (MT-DNIIR) that express a metallothionein promoter driven truncated TβRII which results in a dominant negative mutant that blocks the TGFβ pathway in the prostate. Two founder lines revealed focal regions of low grade PIN, which progress to high grade PIN. Aged mice showed significant histological changes including the development of cribriform and invasive cancer (unpublished data). The MT-DNIIR mice show changes mimicking those observed in hPCa. Since TGFβ is negatively regulated by androgens and has been implicated as a mediator of castration-induced cell death in the prostate, we examined the MT-DNIIR mice for the effects of androgen withdrawal on the ability of the prostate to regress.

METHODS: MT-DNIIR mice aged 50 weeks were placed on zinc sulfate in the water prior to castration. Prostates were collected, weighed and examined histologically.

RESULTS: In non-transgenic castrated mice the anterior prostate (AP), dorsolateral prostate (DLP) and ventral prostate (VP) regressed to 41%, 68% and 52% of sham operated controls (100%) respectively by 35 days. The histology associated with these tissues was consistent with that expected after androgen withdrawal. In contrast, the DLP of the MT-DNIIR mice did not demonstrate the involution observed in the control animals. The DLP failed to regress prior to day 14, and by 35 days post castration the DLP had regressed to 81% of sham operated controls (100%). The DLP showed glands containing normal tall, columnar epithelium beside other glands that were clearly atrophic, suggesting that the level of transgene expression is variable throughout the tissue.

CONCLUSIONS: Although the origin of androgen independent PCa in humans remains unknown, this data suggests that disruption of the TGFβ pathway may be a contibuting event to the development of androgen independant disease through the prevention of prostatic regression after androgen ablation.

Support: T.Z. Thomas is a DOD-PCRP Post-doctoral Fellow, and the J.T. Martell Foundation

The American Urological Association 95th Annual Meeting, April 29-May 4, 2000. Atlanta, Georgia. Published in the Supplement to Journal of Urology, 163, No. 4 abstract 123

AUA 2001 Abstract Submitter

96th Annual Meeting
June 2-7, 2001
Anaheim, CA

Current Abstract: 2005174

ROLE OF TGF-β PATHWAY IN PROSTATE CARCINOGENESIS

William H Tu, Tania Z Thomas, Naoya Masumori, Nashville, TN, Taiji Tsukamoto, Sapporo, Japan, Susan Kasper, Richard L Roberts, Harold L Moses, Scott B Shappell, Robert J Matusik, Nashville, TN

Introduction and Objectives: Transgenic mice provide a mammalian in vivo system to elucidate the mechanism of prostate carcinogenesis and to serve as models for testing potential prostate cancer drug therapies. In human prostate cancers, higher tumor grade has been associated with loss of functional Transforming Growth Factor-β(TGF-β) receptor type II. To identify the role of the TGF-β pathway, the bigenic offspring from a cross of two different transgenic animal lines that develop prostatic lesions were studied.

Methods: One transgenic mouse line, 12T-7f, targets expression of the SV40 large T antigen (Tag) to the prostate using the long probasin promoter. The large T antigen has been shown to bind and inactivate two tumor suppressor genes, p53 and Rb. The second transgenic mouse line, MTR-27H, uses the metallothionein promoter to express a truncated type II receptor which results in a dominant negative mutant (DNIIR) that blocks the TGF-β pathway in the prostate. Both lines develop prostatic lesions comparable to human high grade prostatic intraepithelial neoplasia (HGPIN), with more pronounced epithelial proliferation and atypia in 12T-7f. Twelve male offspring aged 12-23 weeks of the bigenic transgenic mouse line (12T-7f X MTR-27H) were studied by gross, histological, and immunohistological examination (Cytokeratin, AR, Tag, Chromogranin). Tissue was collected from the prostate, seminal vesicle, vas deferens, testis, bladder, bulbourethral gland, para-aortic lymph nodes, neck lymph nodes, lumbar spine, liver, lung, kidney, spleen, brain, adrenal, parotid gland, and submandibular gland.

Results: Although the age-matched transgenic mice developed only HGPIN at comparable time points, the bigenic mice developed both HGPIN and invasive prostate cancer (100 % in mice ≥ 16 wks) with both glandular and neuroendocrine (NE) differentiation. Metastatic Tag positive carcinoma, primarily with NE differentiation, was noted in para-aortic lymph nodes, bone, and viscera, including liver and lung (≥ 50 % of mice ≥16 wks).

Conclusions: In contrast to the 12T-7f and DNRII mice that develop only HGPIN at comparable time points, the bigenic offspring develop invasive carcinoma in the prostate with metastases. The TGF-β pathway and p53/RB pathways are important in prostate carcinogenesis. This study demonstrates that cross breeding transgenic mouse lines can generate new phenotypes representing improved models of human prostate cancer. These models will be helpful for the development of drug therapy in the treatment of human prostate cancer.

TGF-β AND PROSTATE CANCER. Robert J. Matusik, William H. Tu, Tania Z. Thomas, Naoya Masumori, Susan Kasper, Richard L. Roberts, Rosa Serra, Scott B. Shappell, and Harold L. Moses, Department of Urologic Surgery, Vanderbilt University Medical Center, Nashville, TN 37232-2765.

Significant correlative evidence has proposed a role for TGFβ ligands in the development of the prostate and progression of prostate cancer (CaP). In humans, increasing CaP grade has been correlated with increasing levels of TGFβ1. As TGFβ1 normally inhibits prostatic cell growth, increased expression of TGFβ1 in CaP has presented a conundrum. It has been hypothesized that if the CaP cells are unable to respond to the inhibitory effects of TGFβ1 yet are over producing this potent immunosuppressor, then active expression of TGFβ1 could be a selective advantage. To block the TGFβ in the prostate, transgenic mice were generated that express a metallothionein (MT) promoter driven truncated TβRII dominant negative (DN) mutant (MT-DNIIR). Examination of the histology of MT-DNIIR prostates revealed focal changes in prostatic morphology at approximately 12 weeks of age that are comparable to low grade prostatic intraepithelial neoplasia (LGPIN) in humans. By 16.5 weeks of age regions of high grade prostatic intraepithelial neoplasia (HGPIN) was present in all animals examined. At 33 weeks, only one mouse prostate showed a local invasion; however, these mice develop defects in the skeleton that prevents keeping them past this age. In parallel, we have developed transgenic mice that target expression of the SV40 large T antigen (Tag) to the prostate using the prostate-specific large probasin promoter (LPB). The Tag protein binds and inactivates two tumor suppressor genes, p53 and Rb, two genes that can be inactivated in late stage CaP and recent reports have identified p53 loss is some HGPIN. The LPB-Tag mice develop HGPIN by 16 weeks and some develop limited invasive cancer after 20 weeks of age. Bigenic males from the MT-DNIIR x LPB-Tag cross were studied in a time course of 12-23 weeks for gross, histological, and immunohistological characteristics. The bigenic mice developed both HGPIN and invasive prostate cancer in 100% of the animal $\geq$ 16 wks with both glandular and neuroendocrine differentiation. Metastatic Tag positive carcinomas, primarily with NE differentiation, were noted in para-aortic lymph nodes, bone, and viscera, including liver and lung ($\geq$ 50 % of mice $\geq$ 16 wks). Using transgenic mouse models, these studies demonstrate that the loss of the TGF-βand p53/RB pathways are important steps in HGPIN progression to prostatic adenocarcinoma (Supported by DOD Prostate Cancer Center PC992022, R01-CA76142, and the Frances Williams Preston Laboratories of the T.J. Martell Foundation).

Symposium Lecture, Endocrine Society Meeting, Denver CO. June 20-23, 2001.

Curriculum Vitae:

Faculty:

- Dr. Simon Hayward, Assistant Professor, Department of Urologic Surgery.
- Dr. Susan Kasper, Assistant Professor, Department of Urologic Surgery.
- Dr. Richard Roberts, M.D., Ph.D., Research Instructor and Molecular Pathology Fellow, Department of Pathology.

Post-doctoral Fellows:

- Dr. Shane Cutler, with Dr. Coffey's laboratory.
- Dr. Ren Jie Jin, with Dr. Matusik's Laboratory.
- Mr. Janni Mirosevich, (Ph.D. to be awarded in August 2001) with Dr. Matusik's Laboratory.
- Ms. Tiina Pitkänen-Arsiola (Ph.D to be awared June 2001) with Dr. Kasper's Laboratory.

Students:

- Mr. William Tu is a MD/Ph.D. student with Dr. Matusik.

BIOGRAPHICAL SKETCHES

Provide the following information for the key personnel listed on the budget page for the initial budget period

NAME	POSITION TITLE
Hayward, Simon W.	ASSISTANT PROFESSOR

EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, and include post-doctoral training.)

INSTITUTION AND LOCATION	DEGREE (IF APPLICABLE)	YEAR(S)	FIELD OF STUDY
Westfield College (University of London)	Bsc (Hons)	1981	Biochem/Biology
Birkbeck College (University of London)	MSc	1984	Biomolecular Organisation
Imperial Cancer Research Fund (London)	PhD	1991	Cell Biology

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past 3 years and to representative earlier publications pertinent to this application. If the list of publications in the last 3 years exceeds 2 pages, select the most pertinent publications. PAGE LIMITATIONS APPLY. DO NOT EXCEED 3 PAGES FOR THE ENTIRE BIOGRAPHICAL SKETCH PER INVESTIGATOR.

RESEARCH AND PROFESSIONAL EXPERIENCE:

1984-1991 Scientific Officer, Laboratory for Metabolic Studies in Cancer, Imperial Cancer Research Fund, Lincoln's Inn Fields, London, England
1991-1992 Scientific Officer, Histopathology Unit, Imperial Cancer Research Fund, London, England
1992-1995 Postdoctoral Fellow, Department of Anatomy, University of California San Francisco
1995-1998 Assistant Research Anatomist, Dept. of Anatomy, University of California, San Francisco
1996-1998 Assistant Research Anatomist, Dept. of Urology, University of California, San Francisco
1998-2001 Assistant Adjunct Professor, Dept. of Urology, University of California, San Francisco
1999-2001 Member, Genitourinary Oncology Research Program, UCSF Comprehensive Cancer Center
August 2001 Assistant Professor, Departments of Urology and Cancer Biology, Vanderbilt University
August 2001 Member, Vanderbilt-Ingram Cancer Center

HONORS AND AWARDS:

SBUR/Merck Young Investigator Award 1998 (Awarded by the Society for Basic Urological Research)

PUBLICATIONS: (selected from a total of 53)

1. Deshpande N, Mitchell IP, Hayward SW, Love S, and Towler JM. [1991] Tumour enzymes and prognosis in transitional cell carcinoma of the urinary bladder: Prediction of risk of progression in patients with superficial disease. J Urol 146:1247-1251.
2. Hayward SW, Del Buono R, Deshpande N, and Hall PA. [1992] A functional model of adult human prostate epithelium. The role of androgens and stroma in architectural organisation and the maintenance of differentiated secretory function. J Cell Sci 102:361-372.
3. Dahiya R, Sikka S, Hellstrom WJG, Hayward SW, Narayan P, Tonagho EA, and Lue TF. [1993] Phenotypic and cytogenetic characterization of a human corpus cavernosum cell line (DS-1). Biochem Mol Biol Int 30:559-569.
4. Dahiya R, Zhang DY, Ho RJ, Haughney PA, Hayward SW, Cunha GR, and Narayan P. [1995] Regression of LnCaP human prostatic tumor xenografts in athymic nude mice by 13-cis-retinoic acid and androgen ablation. Biochem and Mol Biol Int 35:487-498.

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9. Baskin LS, Hayward SW, Young P, Cunha GR. [1996] Ontogeny of the rat bladder: Smooth muscle and epithelial differentiation. Acta Anatomica 155:163-171.
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13. Baskin LS, Sutherland RS, Hayward SW, Thomson AA, and Cunha GR. [1996] Growth factors and receptors in bladder development and obstruction. Laboratory Investigation 75:157-166.
14. Cunha GR, Hayward SW, Dahiya R, and Foster B. [1996] Smooth muscle-epithelial interactions in normal and neoplastic prostatic development. Acta Anatomica 155:63-72.
15. Hayward SW, Rosen MA, and Cunha GR. [1997] Stromal-epithelial interactions in normal and neoplastic prostate. Br J Urol 79:Suppl 2, 18-26.
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17. Baskin LS, Sutherland RS, Thomson AA, Nguyen H-T, Morgan DM, Hayward SW, Hom YK, DiSandro M, and Cunha GR. [1997] Growth factors in bladder wound healing. J Urol 157:2388-2395.
18. Baskin LS, Sutherland RS, DiSandro M, Hayward SW, Lipschutz J, and Cunha GR. [1997] The effect of testosterone on androgen receptors and human penile growth. J Urol 158:1113-1118.
19. Cunha GR, Donjacour AA, and Hayward SW. [1997] Mesenchymal-epithelial interactions in the development of the male reproductive system. In: Microscopic and Reproductive Development: A dynamic approach. PM Motta, ed. Kluwer Academic Publishers, New York, pp 349-359.
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21. Hayward SW. [1998] A simple method for freezing and storing viable tissue fragments. In Vitro, Cell and Devel Biol 34A:28-29.
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26. Hayward SW, Haughney PC, Rosen MA, Greulich KM, Weier H-UG, Dahiya R, and Cunha GR. [1998] Interactions between adult human prostatic epithelium and rat urogenital sinus mesenchyme in a tissue recombination model. Differentiation 63:31-40.
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35. Hayward SW, Donjacour AA, Thomson AA, and Cunha GR. [2000] Endocrinology of the prostate and benign prostatic hyperplasia. In: Endocrinology 4th Edition. L DeGroot and JL Jameson (eds.). WB Saunders, New York, pp 2357-2367.
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37. Wang Y, Cunha GR, and Hayward SW. (In press). In vitro and in vivo models of prostate cancer. American Cancer Society Atlas of Clinical Oncology. PR Carroll ed. (In press).
38. Mitchell SE, and Hayward SW. (In press). Epithelial-mesenchymal interactions in prostate cancer. In: Prostate Cancer: Scientific and Clinical Aspects. Bridging the Gap. PD Abel, E-N Lalani (eds.), Imperial College Press, London. (In press).

BIOGRAPHICAL SKETCHES

Provide the following information for the key personnel listed on the budget page for the initial budget period

NAME	POSITION TITLE
Susan Kasper	ASSISTANT PROFESSOR

EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, and include post-doctoral training.)

INSTITUTION AND LOCATION	DEGREE (IF APPLICABLE)	YEAR(S)	FIELD OF STUDY
University of Manitoba	B.Sc, Honors	1978	Zoology
University of Manitoba	M.Sc.	1981	Physiology
University of Manitoba	Ph.D.	1984	Physiology

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past 3 years and to representative earlier publications pertinent to this application. If the list of publications in the last 3 years exceeds 2 pages, select the most pertinent publications. PAGE LIMITATIONS APPLY. DO NOT EXCEED 3 PAGES FOR THE ENTIRE BIOGRAPHICAL SKETCH PER INVESTIGATOR.

RESEARCH AND PROFESSIONAL EXPERIENCE:

1986 Postdoctorate position, Dana Farber Cancer Institute, Harvard Medical School, Boston, MA

1987-1989 Postdoctorate position, Department of Molecular Medicine, New England Medical Center Hospitals, Boston, MA

1989-1996 Research Associate, Department of Physiology, University of Manitoba, Winnipeg, Manitoba

1996-2000 Research Asst. Professor, Department of Urologic Surgery, Vanderbilt University Medical Center

1996-Present Research Asst. Professor, Department of Cell Biology, Vanderbilt University (Appointment as Assist. Prof. Pending)

1998-Present Board Member, Institutional Animal Care and Use Committee, Vanderbilt University, Nashville, TN

2001-Present Assistant Professor, Department of Urologic Surgery, Vanderbilt University Medical Center, Nashville, TN

HONORS AND AWARDS:

American Urological Association Gallery Best Poster Award 2000
Society for Basic Urologic Research Travel Award, 1997 and 1998
Stowell-Orbison Award, USCAP - Best Poster, 1997
CaP Cure Award, "Progression of prostate cancer to androgen dependence: the role of the androgen receptor and tumor-derived transcription factors," 1996
American Urological Association Gallery Best Poster Award, 1996
American Urological Association Gallery Best Poster Award, 1995
Society for Basic Urologic Research Award (for abstracts Outstanding Science), 1993
Medical Research Council Fellowship Award, 1988
Medical Research Council Fellowship Award, 1986-1987
Drewry Memorial Scholarship and Medal, Faculty of Medicine, University of Manitoba, 1985

PUBLICATIONS:

1. Kasper S, Worsley IG, Rowe JM, Shiu RPC, and Friesen HG, 1982. Chondrocyte growth factor from the human pituitary gland. J Biol Chem 257:5226-5230.
2. Friesen HG, Dean HJ, and Kasper S, 1985. A perspective on growth hormone and growth. In: Human Growth Hormone (Raiti, S., ed) Pergamon Press, Plenum Publ. Co.

3. Rowe JM, Kasper S, and Shiu RPC, 1986. Purification and characterization of a human mammary tumor-derived growth factor. Cancer Research 46:1408-1412.
4. Kasper S, and Friesen HG, 1986. Human pituitary tissue secretes a potent growth factor for chondrocyte proliferation. J Clin Endocrinol Metab 62:70-76.
5. Kasper S, and Friesen HG, 1986. Growth factors: a selected review. In: Growth Hormone (Tolis G, and Ludecke D.K. eds) Raven Press, NY.
6. Goodman RH, Verhave M, Kasper S, Tsukada T, Mandel G, and Fink JS, 1988. Regulation of expression of the human pre-pro VIP gene. In: Perspectives in Neuroendocrinology. (Wass J, and Scanlon M., eds). Springer-Verlag.
7. Fink JS, Verhave M, Kasper S, Tsukada T, Mandel G, and Goodman RH, 1989. The CGTCA sequence motif is essential for biological activity of the vasoactive intestinal peptide gene cAMP - regulated enhancer. Proc Natl Acad Sci USA 85:6662-6666.
8. Cattini PA, Nachtigal MW, Ludwig SM, Klassen ME, Kasper S, and Nickel BA, 1992. Implantation and transfection procedure: use of gene transfer to examine expression and regulation of human placental hormone. In: Neuroendocrine Research Methods. (Greenstein, B.D., ed.).
9. Kasper S, Popescu RA, Torsello A, Vrontakis ME, Ikejiani C, and Friesen HG, 1992. Tissue-specific regulation of vasoactive intestinal peptide messenger ribonucleic acid levels by estrogen in the rat. Endocrinology 130:1796-1801.
10. Leite V, Vrontakis ME, Kasper S, and Friesen HG, 1993. Bromocriptine inhibits galanin gene expression in the rat pituitary gland. Mol Cell Neuroscience 4:418-423.
11. Kasper S, Rennie PS, Bruchovsky N, Sheppard PC, Cheng H, Lin L, Snoek R, and Matusik RJ, 1994. Cooperative binding of the androgen receptor to two DNA sequences is required for androgen induction of the probasin gene. J Biol Chem 269:31763-31769.
12. Snoek R, Rennie PS, Kasper S, Matusik RJ and Bruchovsky N, 1996. Induction of cell-free, in vitro transcription by recombinant androgen receptor peptides. J Steroid Biochem Mol Biol 59 (3-4): 243-250.
13. Yan Y, Sheppard PC, Kasper S, Lin L, Hoare S, Kapoor A, Dodd JG, Duckworth CL, and Matusik RJ, 1997. Large fragment of the probasin promoter targets high levels of transgene expression to the prostate of transgenic mice. Prostate 32(2):129-139.
14. Metts JC, Kotkin L, Kasper S, Shyr Y, Adams MC, and Brock JW, 1997. Genital malformations and coexistent urinary tract or spinal anomalies in patients with imperforate anus. J Urology 158:1298-1300.
15. Bai G, Kasper S, Matusik RJ, Rennie PS, Moshier JA, and Krongrad A, 1998. Androgen regulation of the human ornithine decarboxylase promoter in prostate cancer cells. J Androl, 19(2): 127-135.
16. Kasper S, Sheppard PC, Yan Y, Pettigrew N, Borowsky AD, Prins GS, Dodd JG, Duckworth ML and Matusik RJ, 1998. Development, progression and androgen-dependence of prostate tumors in probasin-large T antigen transgenic mice: A model for prostate cancer. Laboratory Investigations. Vol. 78, No. 3, p. 319-333. (Erratum, June 1998).
17. Lareyre JJ, Mattei M-G, Kasper S, Ong DE, Matusik RJ and Orgebin-Crist M-C, 1998. Genomic organization and chromosmallocalization of the murine epididymal retinoic acid binding protein (mE-RABP) gene. Mol Reprod Dev, 50, 387-395.
18. Lareyre JJ, Zheng WL, Zhao GQ, Kasper S, Newcomer ME, Matusik RJ, Ong DE and Orgebin-Crist MC, 1998. Molecular cloning and hormonal regulation of a murine epididymal retinoic acid-binding protein messenger ribonucleic acid. Endocrinology, 139 (6), 2971-2981.
19. Snoek R, Bruchovsky N, Kasper S, Matusik RJ, Gleave M, Sato N, Mawji NR, Rennie PS, 1998. Differential transactivation by the androgen receptor in prostate cancer cells. The Prostate, 36, 256-263.

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21. Lareyre JJ, Thomas TZ, Zheng W-L, Kasper S, Ong DE, Matusik RJ, and Orgebin-Crist M-C, 1999. A 5 kilobase pair promoter fragment of the murine epididymal retinoic acid-binding protein gene drives the tissue-specific, cell-specific, and androgen-regulated expression of a foreign gene in the epididymis of transgenic mice. J Biol Chem 274(12): 8282-8290.
22. Kasper S, Rennie PS, Bruchovsky, Lin L, Cheng H, Snoek R, Dahlman-Wright K, Gustafsson J-Å, Shiu, RPC, Sheppard PC, Matusik RJ, 1999. Selective activation of the probasin androgen-responsive region by steroid hormones. J Mol Endocrinology 22:313-325.
23. Shappell SB, Boeglin WE, Olson SJ, Kasper S, Brash AR, 1999. 15-Lipoxygenase-2 (15-LOX-2) is expressed in benign prostatic epithelium and reduced in prostate adenocarcinoma. Am J Pathol 155(1):235-245.
24. Brash AR, Jisaka M, Boeglin WE, Chang MS, Keeney DS, Nanney LB, Kasper S, Matusik RJ, Olson SJ, Shappell S.B, 1999. Investigation of a second 15S-lipoxygenase in humans and its expression in epithelial tissues. Adv Exp Med Biol 469:83-89.
25. Kasper S, Matusik RJ: Rat Probasin, 2000. Structure and function of an outlier lipocalin. (Review) Biochim Biophys Acta 18(1-2):249-258.
26. Matusik RJ, Masumori M, Thomas TZ, Case T, Paul M, Kasper S, Shappell SB, 2000. Transgenic mouse models of prostate cancer. In: Transgenics in Endocrinology, ed. By MM Matzuk, CW Brown, and TR
Kumar. The Humana Press Inc. (Totowa, NJ) In press.
27. Shappell SB, Masumori M, Thomas TZ, Case T, Paul M, Kasper S, Matusik RJ, 2000. Transgenic mouse models of prostate carcinoma: Anatomic, Histopathologic, and molecular considerations. In: Prostate Cancer: Scientific and Clinical Aspects of Bridging the Gap, ed. by PD Abel and E-N Lalani. Imperial College Press (London) In press.
28. Zhang J-F, Thomas TZ, Kasper S, Matusik RJ, 2000. A small composite probasin promoter confers high levels of prostate-specific gene expression through regulation by androgens and glucocorticoids *in vitro* and *in vivo*. Endocrinology 141(12):4698-4710.
29. Lareyre J-J, Reid K, Nelson C, Kasper S, Rennie PS, Orgebin-Crist M-C, Matusik RJ, 2000. Characterization of an androgen-specific response region within the 5' flanking region of the murine epididymal retinoic acid binding protein gene. Biology of Reproduction 63:1881-1892.
30. Lareyre J-J, Winfrey VP, Kasper S, Ong DE, Matusik RJ, Olson GE, and Orgebin-Crist M-C, 2001. Gene duplication gives rise to a new 17 kDa lipocalin that shows epididymal region-specific expression and testicular factors(s) regulation. Endocrinology 142:1296-1308.
31. Masumori N, Thomas TZ, Chaurand P, Case T, Paul M, Kasper S, Caprioli R, Tsukamoto T, Shappell S, Matusik RJ, 2001. A probasin-large T antigen transgenic mouse line develops prostate adeno- and neuroendocrine-carcinoma having metastatic potential. Cancer Res. 61(5):2239-2249.

BIOGRAPHICAL SKETCHES

Provide the following information for the key personnel listed on the budget page for the initial budget period

NAME	POSITION TITLE
Richard L. Roberts, M.D., Ph.D.	RESEARCH INSTRUCTOR

EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, and include post-doctoral training.)

INSTITUTION AND LOCATION	DEGREE (IF APPLICABLE)	YEAR(S)	FIELD OF STUDY
University of Iowa	B.S.	1982	
University of Iowa	M.D.	1987	
University of Iowa	Ph.D.	1993	Anatomy

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past 3 years and to representative earlier publications pertinent to this application. If the list of publications in the last 3 years exceeds 2 pages, select the most pertinent publications. PAGE LIMITATIONS APPLY. DO NOT EXCEED 3 PAGES FOR THE ENTIRE BIOGRAPHICAL SKETCH PER INVESTIGATOR.

RESEARCH AND PROFESSIONAL EXPERIENCE:

1991-1996 Resident in the Division of Anatomical Pathology, Washington University School of Medicine

1994-1995 Neuropathology Chief Resident in the Division of Neuropathology, Washington University School of Medicine

1996-2000 Research Associate with Dr. Philip Stahl in the Department of Cell Biology and Physiology, Washington University School of Medicine

2001-present Research Instructor, Department of Pathology, Vanderbilt University Medical Center

PUBLICATIONS:

1. Roberts RL, Kessel RG, and Tung HN. "Freeze-fracture images of cells and tissues." Oxford University Press, New York, NY, 1991.
2. Roberts RL, Fine F, and Sandra A. Studies of the mechanism of iron transport across the blood-brain barrier. Ann Neurol, 32:543-550, 1992.
3. Roberts RL, and Sandra A. Coated and noncoated pits internalize insulin in pulmonary artery endothelial cell cultures revealed by label-fracture immunocytochemistry. Tis Cell, 24:603-611, 1992.
4. Pfeiffer J, Wick MJ, Roberts RL, Normark S, and Harding C. Alternative processing of class I molecules in macrophages. Nature, 361:359-361, 1993.
5. Roberts RL, Fine R, and Sandra A. The interaction of a transferrin-peroxidase conjugate with the blood-brain barrier. J Cell Sci, 104:521-533, 1993.
6. Monafo WJ, Haslam DB, Roberts RL, Zaki S, Bellini WJ, and Coffin CM. Disseminated measles infection following vaccination in a child with a congenital immune deficiency. J Pediatrics, 124:273-277, 1993.
7. Roberts RL, and Sandra A. Apical and basal membrane polarity in capillaries isolated from rat epididymal fat. J Anatomy, 182:339-347, 1993.
8. Arribas JR, Clifford DB, Fichtenbaum CJ, Roberts RL, Powderly WG, and Storch GA. Detection of Epstein-Barr virus DNA in cerebrospinal fluid for diagnosis of AIDS-related central nervous system lymphoma. J Clin Micro, 33:1580-1583, 1994.

9. Roberts RL, and Sandra A. Ultrastructural characterization of the interaction of transferring with the capillary endothelial cells of the rat thymus. Tissue and Cell, 26:757-766, 1994.
10. Li G, D'Souza-Schorey C, Barbieri MA, Roberts RL, Klippel A, Williams LT, and Stahl PD. Evidence for phosphatidylinositol 3 kinase as a regulator of endocytosis via activation of rab5. Proc Natl Acad Sci, 92:10207-10211, 1995.
11. Kaufman BA, Francel PC, Roberts RL, Argemond E, Park TS, and Dehner LP. Chondroid chordoma of the lateral skull base. Pediatric Neurosurgery, 23:159-163, 1995.
12. Akins PT, Roberts R, Coxe W, and Kaufman BA. Familial colloid cyst of the third ventricle: Case report and review of associated conditions. Neurosurgery, 38:392-395, 1996.
13. Barbieri MA, Roberts R, Muhodadihyay A, and Stahl PD. Rab5 regulates the dynamics of endosome fusion. Bio Cell, 20:331-338, 1996.
14. Muhopadihyay A, Barbieri MA, Funato K, Roberts R, and Shahl PD. Sequential actions of rab5 and rab7 regulate endocytosis in Xenopus oocytes. J Cell Bio, 136:1227-1235, 1997.
15. Alvarez-Dominguez C, Roberts R, and Stahl PD. Internalized listeria monocytogenes modulates intracellular trafficking and delays maturation of the phagosome. J Cell Sci, 110:731-740, 1997.
16. Roberts RL, Barbieri MA, and Stahl PD. Endosome fusion and tubule formation in cells over-expressing GFP-rab5 fusion proteins. Microscopy and Microanalysis, 3(S2):137-138, 1997.
17. Barbieri MA, Hoffenberg S, Roberts R, Mukhopadhyay A, Pomrehn A, Dickey BF, and Stahl PD. Evidence for a symmetrical requirement for Rab5-GTP in vitro endosome-endosome fusion. J Biol Chem, 273:25850-25855, 1998.
18. Teng H, Cole JC, Roberts RL, and Wilkinson RS. "Endocytic active zones": Hot spots for endocytosis in vertebrate neuromuscular terminals. J Neurosci, 19:4855, 1999.
19. Roberts RL, Barbieri MA, Pryse K, Chua M, Morasaki J, and Stahl PD. Endosome fusion in living cells overexpressing GFP-rab5. J Cell Sci, 112:3667, 1999.
20. Roberts RL, Barbieri MA, Ulrich Y, and Stahl PD. Dynamics of GFP-rab5a activation in endocytosis and phagocytosis. J Leukoc Biol 68:627-632, 2000.
21. Barbieri MA, Roberts RL, and Stahl PD. Regulators and effectors of small GTPases: Measurement of the Rab5 PKB/AKT and regulation of Ras activated endocytosis. (W.E. Balch, Channing J, and Hall A, eds) Methods ini Enzymology, In press.
22. Barbieri MA, Roberts RL, Gumusboga A, Highfield H, wells A, and Stahl PD. Epidermal growth factor and receptor trafficking. EGF receptor activation of endocytosis requires rab5a. J Cell Biol, 151:539-548, 2000.

BIOGRAPHICAL SKETCHES

Provide the following information for the key personnel listed on the budget page for the initial budget period

NAME	POSITION TITLE
Ned Shane Cutler	POST-DOCTORAL RESEARCH FELLOW

EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, and include post-doctoral training.)

INSTITUTION AND LOCATION	DEGREE (IF APPLICABLE)	YEAR(S)	FIELD OF STUDY
University of Utah, Salt Lake City, UT	BS	1995	Biology, Chemistry
Duke University, Durham, NC	Ph.D.	2000	Philosophy
Vanderbilt University Medical Center, Dept. of Medicine, Nashville, TN	Post-doctoral training		Medicine

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past 3 years and to representative earlier publications pertinent to this application. If the list of publications in the last 3 years exceeds 2 pages, select the most pertinent publications. PAGE LIMITATIONS APPLY. DO NOT EXCEED 3 PAGES FOR THE ENTIRE BIOGRAPHICAL SKETCH PER INVESTIGATOR.

RESEARCH AND PROFESSIONAL EXPERIENCE:

1993-1993 Student Researcher, Inhalation Toxicology Research Institute, Albuquerque, NM.

1993-1995 Laboratory Technician, Dept. of Pharmacology, University of Utah, Salt Lake City, UT

1995-1995 Rotating Student, Laboratory of Shirish Shenolikar, Dept. of Pharmacology, Duke University, Durham, NC

1995-2000 Graduate Student, Laboratory of Dr. Joseph Heitman, Dept. of Genetics, Duke University, Durham, NC

2000-present Post-doctoral Research Fellow, Laboratory of Dr. Robert Coffey, Dept. of Medicine, Vanderbilt University Medical Center, Nashville, TN

HONORS AND AWARDS:

Vice-Chancellor's Recruitment Incentive for Excellence 2000 (Vanderbilt University), Travel Award 1997-1998-1999-2000 (Duke University Graduate School), Travel Award 1996 (American Society for Microbiology), Membership FKF Honor Society 1995, Award of Merit 1994 (Mountain West Society of Toxicology), Student Research Fellowship 1993 (US Dept. of Energy), University of Utah President's Award 1990-1993-1994-1995, Seville Flowers Scholarship, 1989-95 (Dept. of Biology, University of Utah)

PUBLICATIONS:

1. Nichols WK, Terry CM, Cutler NS, Appleton ML, Jesthi PK, and Yost GS. Oxidation at C-1 controls cytotoxicity of DDD by rabbit and human lung cells. Drug Metabolism and Disposition 23:595-599, 1995.
2. Cutler NS, Heitman J, and Cardenas ME. STT4 is an essential phosphatidylinositol 4-kinase that is a target of worthmannin in *Saccharomyces cerevisiae*. J Biol Chem 272:27671-27677, 1997.
3. Cardenas ME, Sanfridson A, Cutler NA, and Heitman J. Signal-transduction cascades as targets for therapeutic intervention by natural products. Trends in Biotech 16:427-433, 1998.
4. Cutler NS, Heitman J, and Cardenas ME. TOR kinase homologs function in a signal transduction pathway that is conserved from yeast to mammals. Mol and Cell Endocrin 155:135-142, 1999.
5. Cardenas ME, Cutler NS, Lorenz MC, Di Como CJ, and Heitman J. The TOR signaling cascade regulates gene expression in response to nutrients. Genes and Devel 13(24):3271-3279, 1999.
6. Lorenz MC, Cutler NS, and Heitman J. Characterization of alcohol-induced filamentous growth in *Saccharomyces cerevisiae*. Mol Biol of the Cell 11:183-199, 2000.

7. Gsrlach J, Fox DS, Cutler NS, Cox GM, Perfect JR, and Heitman J. Identification and characterization of a highly conserved calcineurin binding protein, CBP1/calcipressin, in *Cryptococcus neoformans*. EMBO J 19(14):3618-3629, 2000.
8. Cutler NS. Rapamycin and other natural products affect regulation of the growth and differentiation of *Saccharomyces cerevisiae*. (Dissertation), 2000.
9. Cutler NS, Cardenas M, Di Como C, Rohde J, and Heitman J. A TOR kinase signaling pathway regulates filamentous growth in *Saccharomyces cerevisiae* and pathogenic fungi. (In preparation).

Research and Professional Experience. Page Limitations Apply. Do not exceed 3 pages for the entire biographical sketch per investigator.

BIOGRAPHICAL SKETCHES

Provide the following information for the key personnel listed on the budget page for the initial budget period

NAME	POSITION TITLE
Ren Jie Jin	UROLOGY FELLOW

EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, and include post-doctoral training.)

INSTITUTION AND LOCATION	DEGREE (IF APPLICABLE)	YEAR(S)	FIELD OF STUDY
Southeast University College of Medicine, Nan Jing, China	M.D.	1985	Doctor of Medicine
Seoul National University, Postgraduate School, Seoul, Korea	M.S.	1999	Science in Medicine
Seoul National University, Postgraduate School, Seoul, Korea	Ph.D.	2001	Philosophy in Medicine

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past 3 years and to representative earlier publications pertinent to this application. If the list of publications in the last 3 years exceeds 2 pages, select the most pertinent publications. PAGE LIMITATIONS APPLY. DO NOT EXCEED 3 PAGES FOR THE ENTIRE BIOGRAPHICAL SKETCH PER INVESTIGATOR.

RESEARCH AND PROFESSIONAL EXPERIENCE:

1985-1996 M.D., Department of Urology, Ji Lin Railway Central Hospital, Ji Lin, China

1997-1998 Research Assistant, Cancer Research Center, Seoul National University College of Medicine, Seoul, Korea

1998-2001 Research Assistant, Clinical Research Institute, Seoul National University College of Medicine, Seoul, Korea

2001-May Urology Fellow, Department of Urologic Surgery, Vanderbilt University Medical Center, Nashville, TN

PUBLICATIONS:

1. Jeong H, Jin RJ, Chung JS, Kwak C, Kim DY, Lee SB, Lee SE. The study for chromosome 3p loss in renal cell carcinoma by fluorescence in situ hybridization using paraffin-embedded tissue. The Korean Journal of Urology, 40(6):697-702, 1999.
2. Lee SE, Jin RJ, Lee SG, Yoon SJ, Park MS, Heo DS, Choi H. Development of a new plasmid vector with PSA-promoter and enhancer expressing tissue-specificity in prostate carcinoma cell lines. Anticancer Research 20(1A):417-422, 2000.
3. Jin RJ, Kwak C, Lee SG, Lee CH, Chung JS, Park MS, Lee E, Lee SE. The application of an anti-angiogenic gene (thrombospondin-1) in the treatment of human prostate cancer xenografts. Cancer Gene Therapy Vol 7:1537-1542, 2000.
4. Jin RJ, Chung JS, Kwak C, Lee CH, Park MS, Lee SE. The effect of clusterin in cisplatin-induced apoptosis on bladder cancer cells. (In press).

Research and Professional Experience. Page Limitations Apply. Do not exceed 3 pages for the entire biographical sketch per investigator.

BIOGRAPHICAL SKETCHES

Provide the following information for the key personnel listed on the budget page for the initial budget period

NAME	POSITION TITLE
Janni Mirosevich	POST-DOCTORAL RESEARCH FELLOW

EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, and include post-doctoral training.)

INSTITUTION AND LOCATION	DEGREE (IF APPLICABLE)	YEAR(S)	FIELD OF STUDY
The University of Western Australia	PhD	2001	Surgery
The University of Western Australia		1997	(Hons) Biochemistry
The University of Western Australia	BS	1996	Biochemistry, Chemistry

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past 3 years and to representative earlier publications pertinent to this application. If the list of publications in the last 3 years exceeds 2 pages, select the most pertinent publications. PAGE LIMITATIONS APPLY. DO NOT EXCEED 3 PAGES FOR THE ENTIRE BIOGRAPHICAL SKETCH PER INVESTIGATOR.

RESEARCH AND PROFESSIONAL EXPERIENCE:

1996-2001 Graduate Student, Laboratory of Dr. Bentel, Department of Surgery, The University of Western Australia, Perth, Australia

2001-Sept. Post-doctoral Research Fellow, Department of Urologic Surgery, Vanderbilt University Medical Center, Nashville, TN

HONORS AND AWARDS:

Urological Research Centre Summer Scholarship, 1995-1996
Australian Kidney Foundation Summer Scholarship, 1996
Cancer Foundation of Western Australia Summer Scholarship, 1997
Neville Stanley Bursary for Honours, 1997
Australian Postgraduate Award, 1998-2001
John Leslie and Dorise Barron Post-graduate Freemasons Scholarship, 1999-2001

PUBLICATIONS:

1. **Mirosevich J**, Bentel J, Zeps N, Redmond S, D'Antuono M & Dawkins H. 1999 Androgen receptor expression of proliferating basal and luminal cells in adult murine ventral prostate. *Journal of Endocrinology* **162** 341-350.
2. **Mirosevich J**, Bentel J & Dawkins H. 2000 Regulation of caltrin mRNA expression by androgens in the murine prostate. *Journal of Andrology* In Press.

BIOGRAPHICAL SKETCHES

Provide the following information for the key personnel listed on the budget page for the initial budget period

NAME	POSITION TITLE
Tiina Inkeri Pitkänen-Arsiola	Post-Doctoral Research Fellow

EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, and include post-doctoral training.)

INSTITUTION AND LOCATION	DEGREE (IF APPLICABLE)	YEAR(S)	FIELD OF STUDY
University of Kuopio, Kuopio, Finland		1988	Computer Science & Applied Mathematics
University of Kuopio, Kuopio, Finland	M.Sc.	1990	Natural & Environmental Sciences
University of Kuopio, Kuopio, Finland	Ph.D.	2001	Natural & Environmental Sciences

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past 3 years and to representative earlier publications pertinent to this application. If the list of publications in the last 3 years exceeds 2 pages, select the most pertinent publications. PAGE LIMITATIONS APPLY. DO NOT EXCEED 3 PAGES FOR THE ENTIRE BIOGRAPHICAL SKETCH PER INVESTIGATOR.

RESEARCH AND PROFESSIONAL EXPERIENCE:

1996-present	Supervisor to 7 M.Sc. students, University of Kuopio, Kuopio, Finland
1988-2001	Fee-Paid Teacher, University of Kuopio, Kuopio, Finland
1987	Acting Assistant, Department of Applied Zoology, University of Kuopio, Kuopio, Finland
1988&1989	Researcher, funded by the Ministry of Agriculture and Forestry, University of Kuopio, Kuopio, Finland
1991	Acting Lecturer, Department of Applied Zoology, University of Kuopio, Kuopio, Finland
1991	Acting Senior Assistant, Department of Applied Zoology, University of Kuopio, Kuopio, Finland
1993	Researcher, funded by the Ministry of Education, University of Kuopio, Kuopio, Finland
1993-1995	Researcher, funded by the Ministry of Agriculture and Forestry, University of Kuopio, Kuopio, Finland
1995-1998	Graduate School Researcher, National Graduate School of Fish Biology and Fisheries, University of Kuopio, Kuopio, Finland
1999-2000	Researcher, funded by the National Technology Agency, University of Kuopio, Kuopio, Finland
2000-present	Assistantship in Biotechnology, Institute of Applied Biotechnology, University of Kuopio, Kuopio, Finland
2000-July	Post-Doctoral Research Fellow, Department of Urologic Surgery, Vanderbilt University Medical Center, Nashville, TN

PUBLICATIONS:

1. Mäkinen A, Pitkänen T, and Andersson M., 1997. Two cases of reciprocal translocations in domestic pigs producing small litters. Journal of Animal Breeding and Genetics, 114(5):337-384.
2. Krasnov A, Reinisalo M, Pitkänen TI, Mölsä H, Nishikimi M, 1998. Expression of rate gene for L-gulono-γ-lactone oxidase, the key enzyme of L-ascorbic acid biosynthesis, in guinea pig cells and in teleost fish rainbow trout (*Oncorhynchus mykiss*). Biochimica et Biophysica Acta, 1381:241-248.

3. Pitkänen TI, Krasnov A, Reinisalo M, Mölsä H, 1999. Transfer and expression of glucose transporter and hexokinase genes in salmonid fish. Aquaculture, 173:319-332.
4. Krasnov A, Pitkänen TI, Reinisalo M, Mölsä H, 1999. Expression of human glucose transporter type I and rat hexokinase type II cDNAs in rainbow trout embryos: effect on glucose metabolism. Marine Biotechnology, 1:25-32.
5. Pitkänen TI, Krasnov A, Teerijoki H, Mölsä H, 1999. Transfer of growth hormone (GH) transgenes into Arctic charr (*Salvelinus alpinus* L.) I. Growth response to various GH constructs. Genetic Analysis: Biomolecular Engineering 15:91-98.
6. Krasnov A, Ågren JJ, Pitkänen TI, Mölsä H, 1999. Transfer of growth hormone (GH) transgenes into Arctic charr (*Salvelinus alpinus* L.) II. Nutrient partitioning in rapidly growing fish. Genetic Analysis: Biomolecular Engineering, 15:99-105.
7. Krasnov A, Pitkänen TI, Mölsä H, 1999. Gene transfer for targeted modification of salmonid fish metabolism. Genetic Analysis: Biomolecular Engineering, 15:115-119.
8. Teerijoki H, Krasnov A, Pitkänen TI, Mölsä H, 2000. Cloning and characterization of glucose transporter in teleost fish rainbow trout (*Oncorhynchus mykiss*). Biochimica et Biophysica Acta 1494:290-294.
9. Pitkänen TI, Xie SQ, Krasnov A, Mason P, Mölsä H, Stickland NC, 2001. Changes in tissue cellularity are associated with growth enhancement in genetically modified Arctic charr (*Salvelinus alpinus* L.) carrying recombinant growth hormone gene. Marine Biotechnology (In press).
10. Teerijoki H, Krasnov A, Pitkänen TI, Mölsä H, 2001. Monosaccharide uptake in common carp (*Cyprinus carpio*) EPC cells is mediated by facilitative glucose transporter. Comparative Biochemistry and Physiology (In press).

BIOGRAPHICAL SKETCHES

Provide the following information for the key personnel listed on the budget page for the initial budget period

NAME	POSITION TITLE
William H. Tu	M.D./PH.D. STUDENT

EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, and include post-doctoral training.)

INSTITUTION AND LOCATION	DEGREE (IF APPLICABLE)	YEAR(S)	FIELD OF STUDY
University of Maryland School of Life Sciences, College Park, MD	BS	1998	Biochemistry, Biology, Microbiology (Hons)

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past 3 years and to representative earlier publications pertinent to this application. If the list of publications in the last 3 years exceeds 2 pages, select the most pertinent publications. PAGE LIMITATIONS APPLY. DO NOT EXCEED 3 PAGES FOR THE ENTIRE BIOGRAPHICAL SKETCH PER INVESTIGATOR.

RESEARCH AND PROFESSIONAL EXPERIENCE:

1994-1995	Laboratory Associate, United States Army Medical Research Institute of Infectious Disease, Pathology Division, Frederick, MD
1996	Laboratory Associate, University of Maryland School of Medicine, Baltimore, MD
1997	Nursing Station Volunteer, Shady Grove Adventist Hospital, Orthopedics Unit, Rockville, MD
1995-1998	Student Researcher, University of Maryland, Departments of Microbiology & Entomology, College Park, MD
1998-Present	M.D./Ph.D. Student, Vanderbilt University School of Medicine, Nashville, TN

HONORS AND AWARDS:

Howard Hughes Medical Institute Research Fellowship 1997-1998, American Institute of Chemists Foundation and the District of Columbia Institute of Chemists Student Award Certificate 1998, Senior Merck Index Award 1997, Eastman Kodak Company International Science and Engineering Fair Winner Award 1992

PUBLICATIONS:

1. Kasper S, Tu W, Roberts R, Shappell SS, Matusik RJ, 2001. The LPB-tag transgenic mouse model for prostate cancer. In: Methods in Prostate Cancer Research, ed by P. Jackson and P. Russell. The Humana Press, Inc. (NJ). In press.

MANUSCRIPTS IN PREPARATION:

1. Masumori N, Tu WH, Kasper S, Tsukamoto T, Shappell SB, Matusik RJ. Allograft model of androgen independent prostatic neuroendocrine carcinoma derived from LPB-tag transgenic mouse line.

ABSTRACTS:

1. Matusik RJ, Matusik RJ, Masumori N, Thomas T, Zhang J-F, Tu W, Case T, Paul M, Shappell SB, Kasper S, 2000. New insights into androgen action. Hormones and Cancer 2000 Conference, November 3-7, Port Douglas, Australia.

2. Tu WH, Thomas TZ, Masumori N, Tsukamoto T, Kasper S, Roberts RL, Moses HL, Shappell SB, Matusik RJ. Role of TGF-β pathway in prostate carcinogenesis. The American Urological Association 96th Annual Meeting, June 2-7, 2001. Anaheim, California.

3. Masumori N, Tu WH, Kasper S, Tsukamoto T, Shappell SB, Matusik RJ. Allograft model of androgen independent prostatic neuroendocrine carcinoma derived from LPB-TAG transgenic mouse line. The American Urological Association 96th Annual Meeting, June 2-7, 2001. Anaheim, California.

4. Matusik RJ, Tu WH, Thomas TZ, Masumori N, Kasper S, Roberts RL, Serra R, Shappell SB, Moses HL, 2001. TGF-β and prostate cancer. Symposium lecture, Endocrine Society 83rd Annual Meeting, June 20-23, Denver, Colorado.

Research and Professional Experience. Page Limitations Apply. Do not exceed 3 pages for the entire biographical sketch per investigator.